

We claim:

1. A method for determining active plasminogen activator inhibitor-Type 1 (PAI-1) in a biological fluid, the method comprising the steps of:
 - 5 (i) providing a sample of a biological fluid; and
 - (ii) measuring the amount of PAI-1/multimeric vitronectin complex in the sample to determine active PAI-1 in the sample.
2. The method of claim 1 wherein step (ii) comprises the steps of:
 - 10 (a) contacting the sample either simultaneously or stepwise with a first antibody which binds selectively to PAI-1 and a labelled second antibody which binds selectively to multimeric vitronectin; and
 - (b) determining the second antibody bound to the complex to measure the amount of PAI-1/multimeric vitronectin complex in the sample.
3. The method of claim 1 wherein step (ii) comprises the steps of:
 - 15 (a) contacting the sample either simultaneously or stepwise with a first antibody which binds selectively to multimeric vitronectin and a labelled second antibody which binds selectively to PAI-1; and
 - (b) determining the second antibody bound to the complex to measure the amount of PAI-1/multimeric vitronectin complex in the sample.
4. The method of claim 1 wherein step (ii) comprises the steps of:
 - 20 (a) contacting the sample either simultaneously or stepwise with a first antibody which binds selectively to PAI-1 and a labelled second antibody which binds selectively to multimeric vitronectin;
 - (b) separating the PAI-1/multimeric vitronectin/first antibody/second

antibody complex formed in step (a) from the sample; and

(c) determining the second antibody bound to the complex to measure the amount of PAI-1/multimeric vitronectin complex in the sample.

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5. The method of claim 1 wherein step (ii) comprises the steps of:

(a) contacting the sample either simultaneously or stepwise with a first antibody which binds selectively to multimeric vitronectin and a labelled second antibody which binds selectively to PAI-1;

(b) separating the PAI-1/multimeric vitronectin/first antibody/second antibody complex formed in step (a) from the sample; and

(c) determining the second antibody bound to the complex to measure the amount of PAI-1/multimeric vitronectin complex in the sample.

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6. The method of claim 1 wherein step (ii) comprises the steps of :

(a) simultaneously contacting the sample with a first antibody which binds selectively to PAI-1, the first antibody being immobilised on a solid support, and with a labelled second antibody which binds selectively to multimeric vitronectin ; and

(b) determining the second antibody bound to the solid support to measure the amount of PAI-1/multimeric vitronectin complex in the sample.

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7. The method of claim 1 wherein step (ii) comprises the steps of :

(a) contacting the sample with a first antibody which binds selectively to PAI-1, the first antibody being immobilised on a solid support;

(b) contacting the solid support with a labelled second antibody which binds selectively to multimeric vitronectin ; and

(c) determining the second antibody bound to the solid support to measure the amount of PAI-1/multimeric vitronectin complex in

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the sample.

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8. The method of claim 1 wherein step (ii) comprises the steps of:

(a) simultaneously contacting the sample with a first antibody which binds selectively to multimeric vitronectin, the first antibody being immobilised on a solid support, and with a labelled second antibody which binds selectively to PAI-1; and

(b) determining the second antibody bound to the solid support to measure the amount of PAI-1/multimeric vitronectin complex in the sample.

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9. The method of claim 1 wherein step (ii) comprises the steps of:

(a) contacting the sample with a first antibody which binds selectively to multimeric vitronectin, the first antibody being immobilised on a solid support;

(b) contacting the solid support with a labelled second antibody which binds selectively to PAI-1; and

(c) determining the second antibody bound to the solid support to measure the amount of PAI-1/multimeric vitronectin complex in the sample.

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10. The method of claim 1 wherein step (ii) comprises the steps of:

(a) contacting the sample with a first antibody which binds selectively to PAI-1, the first antibody being immobilised on a solid support;

(b) contacting the solid support with a second antibody which binds selectively to multimeric vitronectin;

(c) contacting the solid support with a labelled third antibody which binds selectively to the second antibody; and

(d) determining the third antibody bound to the solid support to measure the amount of PAI-1/multimeric vitronectin complex in the sample.

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11. The method of claim 1 wherein step (ii) comprises the steps of:

- contacting the sample with a first antibody which binds selectively to multimeric vitronectin, the first antibody being immobilised on a solid support;
- contacting the solid support with a second antibody which binds selectively to PAI-1;
- contacting the solid support with a labelled third antibody which binds selectively to the second antibody; and
- determining the third antibody bound to the solid support to measure the amount of PAI-1/multimeric vitronectin complex in the sample.

12. The method of claim 1 wherein step (ii) comprises the steps of:

- contacting the sample, either simultaneously or stepwise, with a first antibody which binds selectively to PAI-1 and to which is attached one member of a capture pair and with a labelled second antibody which binds selectively to multimeric vitronectin to form a mixture;
- contacting the mixture with a solid support on which is immobilised the other member of the capture pair; and
- determining the second antibody bound to the solid support to measure the amount of PAI-1/multimeric vitronectin complex in the sample.

13. The method of claim 1 wherein step (ii) comprises the steps of:

- contacting the sample either simultaneously or stepwise, with a first antibody which binds selectively to multimeric vitronectin and to which is attached one member of a capture pair and with a labelled second antibody which binds selectively to PAI-1 to form a mixture;
- contacting the mixture with a solid support on which is

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5 (c) immobilised the other member of the capture pair; and determining the second antibody bound to the solid support to measure the amount of PAI-1/multimeric vitronectin complex in the sample.

14. The method of any one of claims 1 to 13 wherein the biological fluid is selected from the group consisting of whole blood, plasma, serum, urine, saliva, amniotic fluid, cerebrospinal fluid and a tissue extract.

10 15. The method of any one of claims 1 to 13 wherein the biological fluid is whole blood, plasma or serum.

16. The method of any one of the preceding claims wherein the second antibody is labelled with a directly detectable label.

15 17. The method of any one of the preceding claims wherein the second antibody is labelled with a component of a signal-generating system.

18. The method of claim 17 wherein the component is an enzyme selected
20 from the group consisting of alkaline phosphatase, amylase, luciferase, catalase, beta-galactosidase, glucose oxidase, glucose-6-phosphate dehydrogenase, hexokinase, horseradish peroxidase, lactamase, urease and malate dehydrogenase.

25 19. The method of any one of claims 1 to 15 wherein the second antibody is labelled with a fluorophore.

20. The method of claim 19 wherein the fluorophore is selected from the group consisting of a coumarin, a rare earth metal ion, chelate or chelate complex, a fluorescein, rhodamine and a rhodamine derivative.

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21. The method of any one of claims 1 to 15 wherein the second antibody is labelled with a luminescent material.

22. The method of claim 21 wherein the luminescent material is selected 5 from the group consisting of a cyclic diacyl hydrazide, luminol, isoluminol, an acridinium ester, a pyridopyridazine, a dioxerane, a bioluminescent protein and a luciferase.

23. The method of any one of claims 1 to 15 wherein the second antibody 10 is labelled with a label selected from the group consisting of a metal complex, a stable free radical, a vesicle, a liposome, a colloidal particle, a latex particle, a spin label and biotin/avidin.

24. The method of any one of claims 6 to 13 wherein the solid support is 15 selected from the group consisting of an ELISA plate, a polyacrylamide matrix, a polystyrene tube, polystyrene beads, latex particles, paramagnetic particles, acrylic particles and gelatin particles.

25. A kit for determining active PAI-1 in a biological fluid comprising:
20 (a) a first antibody which binds selectively to PAI-1; and
 (b) a labelled second antibody which binds selectively to multimeric vitronectin.

26. A kit for determining active PAI-1 in a biological fluid comprising:
25 (a) a first antibody which binds selectively to multimeric vitronectin;
 and;
 (b) a labelled second antibody which binds selectively to PAI-1.

27. The kit of claim 25 or 26 wherein said first antibody is immobilised on a 30 solid support.

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8. The kit of any one of claims 25 to 27 further comprising a set of calibration standards.

29. A kit for determining active PAI-1 in a biological fluid comprising:

5 (a) a first antibody which binds selectively to PAI-1;

(b) a second antibody which binds selectively to multimeric vitronectin; and

(c) a labelled third antibody which binds selectively to said second antibody.

10 30. The kit of claim 29 wherein said first antibody is immobilised on a solid support.

15 31. The kit of claim 29 or 30 further comprising a set of calibration standards.

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